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Serological survey for canine angiostrongylosis in Slovakia

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Summary

In recent years *Angiostrongylus vasorum* has become another important heart parasite of dogs besides *Dirofilaria immitis*, with intense spread into new areas of Europe. The first two cases of canine angiostrongylosis in Slovakia were observed in 2013, demonstrating that this life-threatening parasitic disease of dogs has expanded into this territory too. One year after the first *A. vasorum* findings, a serological survey was conducted to assess the current distribution of this parasitic infection in dogs from Slovakia.
Serum samples from 225 dogs were collected from 29 veterinary practices situated in 22 districts of Slovakia and tested by ELISA for the presence of circulating *A. vasorum* antigens and additionally for the detection of specific antibodies against *A. vasorum*.
Fourteen samples (6.22 %) were seropositive in at least one ELISA. Of these, 7 dogs (3.11 %) were only antibody-positive and 4 dogs (1.78 %) were positive only for circulating *A. vasorum* antigen. Three animals out of 225 examined (1.33 %) were positive in both ELISAs.
Keywords: *Angiostrongylus vasorum*; dogs; ELISA; antigen and antibody detection; seroprevalence; Slovakia

Introduction

Canine angiostrongylosis, caused by the metastrongylid nematode *Angiostrongylus vasorum*, was established in dogs for a long time in endemic areas of France, Denmark, southern England, Wales and Ireland. In more recent years its spread into new areas of Europe has been demonstrated. The first two autochthonous cases of canine angiostrongylosis in Slovakia were observed in 2013 (Hurníková *et al*., 2013; Miterpáková *et al*., 2014), showing that this life-threatening parasitic disease of dogs has expanded into this territory too.
Given that the parasite has also been diagnosed in dogs in the neighbouring countries Hungary (e.g. Majoros *et al*., 2010; Tolnai *et al*., 2015) and the Czech Republic (Hajnalová *et al*., 2014), and also during a serological survey in Poland (Schnyder *et al*., 2013b), a serological survey was performed to assess the current distribution of this parasitic infection in dogs from Slovakia.

Materials and Methods

Serum samples from 225 dogs were collected from 29 veterinary practices situated in 22 districts of Slovakia. The veterinarians were asked to choose dogs randomly, and individual data related to age, breed, sex, locality of residence, living habitat and health status were collected in a questionnaire. All samples were delivered to the Institute of Parasitology (Slovak Academy of Sciences, Košice, Slovakia).
Serum samples were tested by ELISA for the presence of circulating *A. vasorum* antigens using mono-and polyclonal antibodies as previously described, with a sensitivity of 95.7 % and a specificity of 98.8 % (Schnyder *et al*., 2011), and additionally for the detection of specific antibodies against *A. vasorum* in a sandwich-ELISA using *A. vasorum* adult somatic antigen purified by monoclonal antibodies (mAb Av 5/5), with a sensitivity of 81.0 % and a specificity of 98.8 % (Schucan *et al*., 2012), at the Institute of Parasitology,

Table 1. Dog sera from Slovakia (n=225) tested for the presence of circulating *Angiostrongylus vasorum* antigen and specific antibodies against *Angiostrongylus vasorum*

n=225	Number positive	% (95 % confidence interval)
Total seropositive samples	14	6.22 (±3.16)
Seropositive for antibodies and antigen	3	1.33 (±1.5)
Seropositive for antibody	10	4.44 (±2.69)
Seropositive for antibodies only	7	3.11 (±2.27)
Seropositive for antigen	7	3.11 (±2.27)
Seropositive for antigen only	4	1.78 (±1.73)

University of Zurich, Switzerland. All test runs included a background control, a conjugate control, three positive control sera from three experimentally infected dogs and two negative control sera from uninfected dogs.
The collected data were analyzed by a geographic information system (GIS) using the program RegioGraph 10 (GfK GeoMarketing, Bruchsal, Germany) to visualize the regional distribution of collected and analyzed serum samples and *A. vasorum* antigen- and/or antibody-positive samples. The locations of positive samples were displayed on maps with administrative and postcode boundaries based on the two-digit postcodes of Slovakia as points of reference.
The seroprevalence values were calculated with 95 % confidence intervals (CI) using the STATISTICA 6 Base programme (StatSoft, Inc., 2001).

Results

The serological results of all tested samples are summarized in Table 1. Fourteen samples (6.22 %; CI ± 3.16 %) were seropositive in at least one ELISA. Of these, 7 dogs (3.11 %) were only antibody-positive and 4 dogs (1.78 %) were positive only for circulating *A. vasorum* antigen. Three animals out of 225 examined (1.33 %) were positive in both ELISAs.
Seropositive dogs came from different regions of Slovakia, with the highest accumulation in the south-western part (Fig. 1). All three dogs positive for both circulating antigens and specific antibodies against *A. vasorum* lived in the Bratislava region – two in the city of Bratislava and one in the Austrian village of Deutsch Jahrndorf on the border between Hungary and Slovakia, roughly 20 km from Bratislava.

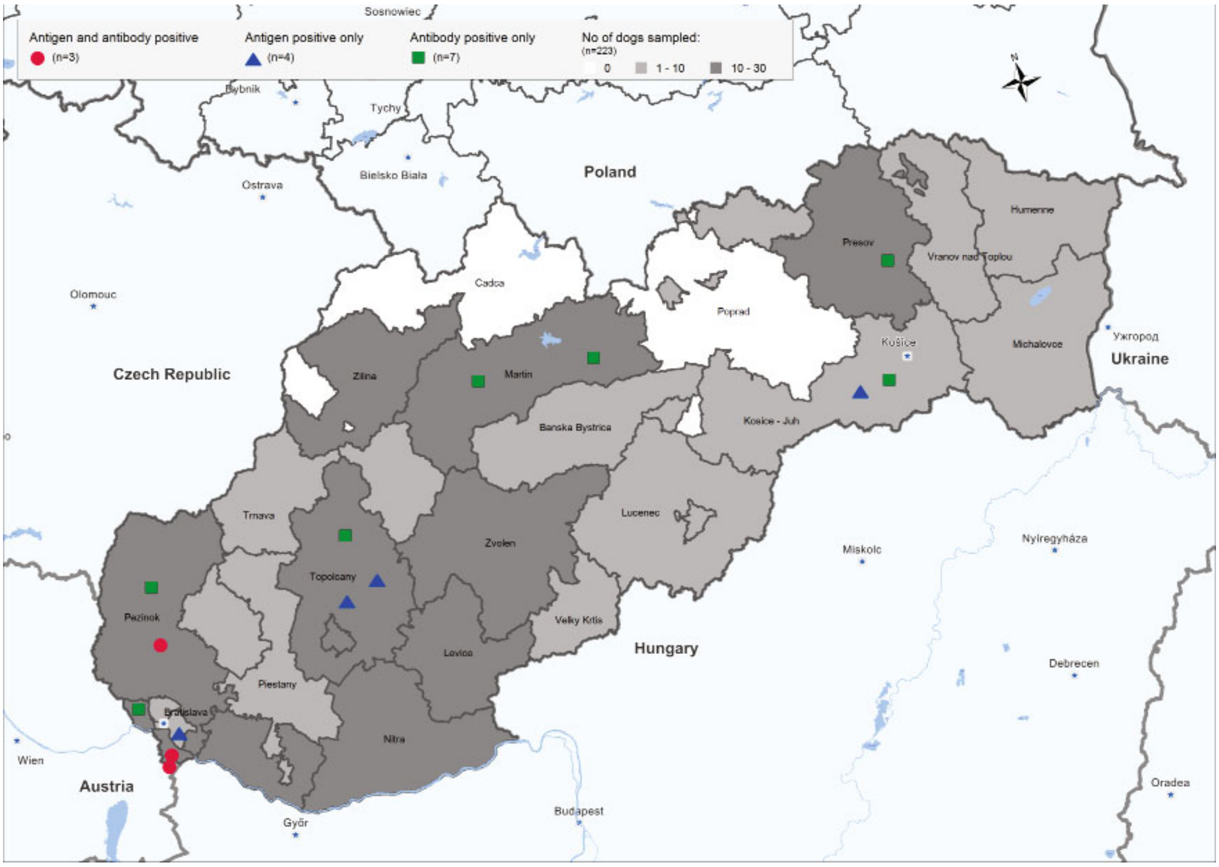

Figure 1. Distribution of dogs serologically positive for *Angiostrongylus vasorum* in Slovakia

Table 2. Epidemiological and anamnestic data of 14 dogs seropositive for *Angiostrongylus vasorum*

Sample no.	*Serological result	Breed	Age	Gender	**Dog's residence	Kept	Dog-walking	Unusual feeding habits	Health problems
1	Ag-positive	Akita-Inu	5 years	female	SE	outdoors	off-leash	dirt-devouring	unknown
2	Ab-positive	Miniature Schnauzer	5 years	male	SW	indoors	off-leash	dirt-devouring	unknown
3	Ag-positive	Giant Schnauzer	3 years	female	SW	outdoors	off-leash	unknown	apathy, anaemia, appetite loss
4	Ag-positive	Newfoundland	3 years	male	CW	outdoors	off-leash	grass-eating	unknown
5	Ab-positive	Weimaraner	1 year	male	CW	outdoors	off-leash	grass-eating	unknown
6	Ab-positive	crossbreed	3 years	female	NE	indoors	off-leash	grass-eating	unknown
7	Ag-positive	crossbreed	2 years	female	CW	indoors	off-leash	dirt-devouring grass-eating	unknown
8	Ab-positive	Maltese	5 years	female	SE	indoors	on-leash	grass-devouring	appetite and weight loss
9	Ab-positive	Dogo Argentino	5 years	male	SW	outdoors	off-leash	unknown	unknown
10	Ag- and Ab-positive	unlisted	unlisted	male	SW	indoors	off-leash	grass-eating	apathy, diarrhoea, pyrexia
11	Ag- and Ab-positive	Central Asian Shepherd Dog	8.5 years	female	Austria	outdoors	off-leash	grass-eating	limp
12	Ab-positive	Yorkshire Terrier	7 years	male	NC	indoors	off-leash	grass-eating	epileptic seizures
13	Ag- and Ab-positive	Shih Tzu	5 years	male	SW	indoors	off-leash	grass-eating	unknown
14	Ab-positive	Leonberger	3 years	male	NC	outdoors	off-leash	grass-eating	unknown

* Ag – Antigen, Ab – Antibody
** Regions of Slovakia: SE – south-east, SW – south-west, NE – north-east, CW – central-west, NC – north-central

The analysis of epidemiological and anamnestic data contained in the questionnaires showed that the average age of seropositive dogs was 4.3 years with a range from 1 to 8.5 years. Among the positive dogs, large and small breeds were equally represented, and 8 individuals were males and 6 females. Seven of the dogs were kept outdoors and 7 indoors, and all owners but one reported that the dog moved outside mostly without restraint and leash. In ten of the seropositive dogs, grass feeding and also devouring other components in the environment, including waste and excreta, were frequently observed (Table 2).

From the medical point of view, 9 out of 14 seropositive dogs showed no clinical signs, but in three seropositive dogs signs potentially related to the *A. vasorum* infection were registered, including significant weight loss and lack of appetite, lethargy, epileptic seizures, anaemia, diarrhoea, and pyrexia.

Discussion

The serological survey on canine angiostrongylosis presented here was conducted one year after the first findings of *Angiostrongylus vasorum* in Slovakia confirmed by larval isolation with Baermann's method (Hurníková *et al*., 2013; Miterpáková *et al*., 2014). Our results indicated relatively high overall seropositivity (6.22 %), with *A. vasorum* circulating antigens and/or specific antibodies against the parasite being diagnosed in 14 of the 225 dogs examined. In total, 1.33 % of the dogs (3/255, CI ± 1.5 %) were positive in both ELISAs. By comparison, in a known endemic area such as southern England, only 0.97 % of the dogs were seropositive for both ELISAs (Schnyder *et al*., 2013a). However, it needs to be emphasized that in that study more than 4,000 serum samples were tested. The results presented here are comparable with a recent serosurvey performed in Portugal, where the parasite was also discovered only recently: out of a total of 341 shelter dogs, 1.17 % were seropositive for both ELISAs (Alho *et al*., 2014).

Although angiostrongylosis is considered to be an infection that more frequently affects dogs younger than one year (Chapman *et al*., 2004; Koch *et al*., 2005; Staebler *et al*. 2005; Barutzki and Schaper, 2009; Chapman *et al*., 2004; Staebler *et al*., 2005), our results confirmed that older individuals are also at risk. Interestingly, one single dog tested antibody-positive at the age of one year, while all others were older than 3 years and in particular the oldest dog, positive for the presence of both, antigens and antibodies, was 8.5 years old at the time of sampling (Table 2). Since *A. vasorum* infections may persist for a lifetime in untreated dogs (Guilhon and Cens, 1969) and occasionally remain unnoticed for a long time period (due to restricted or nonspecific clinical signs (Schnyder *et al*., 2010) or lack of disease awareness, especially in new endemic areas where *A. vasorum* has not previously been diagnosed), the time point of oral intake of the infectious stages in naturally infected dogs is unknown. Therefore, the time point at which the positive dogs in this study were infected is also unknown and may have occurred years before and/or on multiple different occasions.

In epidemiological terms, the relevant information is that all dogs but one were moving outdoors freely without leashes, and except for two, all owners of seropositive dogs reported that their dog often ate grass or other environmental components. Given that the parasite's life cycle is indirect via snails (and possibly their secreta) (Ferdushy and Hasan, 2010) and perhaps paratenic hosts (Bolt *et al*., 1993; Mozzer and Lima, 2015), these feeding habits should be considered to be a major risk factor.

Regarding breed predisposition for angiostrongylosis, results from previous studies are inconclusive. Some authors did not report any contingency (Koch and Willesen, 2009; Staebler *et al*., 2005), while others suggest that the risk may be higher in purebred dogs or in the hunting dog breeds (Chapman *et al*., 2004; Conboy, 2004). Among the seropositive dogs detected within our study, 7 belonged to large breeds, 3 were small breeds and 3 individuals were of unascertained identity, confirming the trend of no breed predisposition.

From a clinical point of view it can be very difficult to identify clinical signs unambiguously related to *A. vasorum* infection, since the clinical picture of this disease is very diverse and can evoke a variety of other cardiopulmonary, neurological or haematological disorders or further nonspecific signs (Koch and Willesen, 2009; Capogna *et al*., 2012). In fact, the owners of three serologically positive dogs reported signs such as apathy, anaemia, and marked loss of weight and appetite, which are nonspecific but quite common in dogs infected with *A. vasorum* (Koch and Willesen, 2009).

The above-mentioned broad spectrum of clinical signs and a possible, at least initially, asymptomatic course of the disease may be reasons why veterinary practitioners may overlook the infection in patients, especially in areas where it has not previously occurred. Since standard microscopic methods for the isolation and identification of first larval stages of lungworms are actually rarely implemented in veterinary practices, a diagnosis may be missed, and more specific methods such as the Baermann funnel method need to be applied. Alternatively, a new commercially available antigen blood test (AngioDetect®, Idexx Laboratories, USA) may be used, with which results can be obtained within 15 minutes. This test can detect an ongoing infection starting from 9 weeks after infection with a very high specificity of 100 % (CI: 97.6 – 100 %) (Schnyder *et al*., 2014), whereas the ELISAs employed in this study detect circulating antigens around 5 weeks post infection (Schnyder *et al*., 2011) and specific antibodies starting from 3 weeks post infection (Schucan *et al*., 2012), both also with very high specificity (98.8 %). In all three tests, including the two ELISAs used in this study, cross-reactivity against several helminthes, and in particular against the widespread lung worm *Crenosoma vulpis* and the less common heart worm *Dirofilaria immitis* was evaluated, delivering these high specificities. On the other hand, specific antibodies against *A. vasorum* and circulating antigens can persist for more than 9 and 4 weeks, respectively, after treatment (Schnyder *et al*., 2011; Schucan *et al*., 2012). Therefore, while the antigen-based rapid assay has a primary application in the diagnosis of dogs clinically suspect for canine angiostrongylosis, ELISA-based techniques are important tools for mass screening and monitoring of infections in non-endemic or newly infested regions. In particular in areas of low prevalence, the combination of both ELISAs for the detection of *A. vasorum* antigen and specific antibodies is of great value in terms of positive predictive value (Schnyder *et al*., 2013a).

A number of factors influence the distribution of *A. vasorum* (Morgan *et al*., 2009). The parasite does not necessarily occur in large endemic areas, like *Dirofilaria* spp., but its presence is incoherent with hyperendemic foci surrounded by areas of low prevalence or localities with sporadic cases (Barutzki and Schaper, 2009; Guardone *et al*., 2013; Schnyder *et al*., 2013b). However, it is clear from recent studies that the geographic range of *A. vasorum* in Europe is changing and that the parasite has been identified in new areas distant from the original endemic foci in recent years. One of the new regions in which the occurrence of canine angiostrongylosis can be expected in the very near future is Central and Eastern Europe. In addition to Slovakia, serological screening was carried out in Poland in 3,345 dogs, revealing 1.79 % of them to be antibody-positive and 0.51 % concurrently antigen- and antibody-positive (Schnyder *et al*., 2013b). In Poland, which neighbours Slovakia to the north, *A. vasorum* larvae were also confirmed in wolves using Baermann's method (Szczęsna *et al*., 2007), and adult parasites were detected in the pericardium and pulmonary arteries of four foxes (Demiaszkiewicz *et al*., 2014). In Hungary, which borders Slovakia to the south, the presence of *A. vasorum* was confirmed in a dog and in the intermediate host *Arion lusitanicus* (Portuguese slug) in 2010 (Majoros *et al*., 2010) and also in wild carnivores such as foxes (Sreter *et al*., 2003) and golden jackals (Takacs *et al*., 2014) and is meanwhile broadly spread, also in the neighboring areas (Tolnai *et al*., 2015). In the Czech Republic, to the north-west of Slovakia, the first case of canine angiostrongylosis was reported in 2014 (Hajnalová *et al*., 2014). In Austria only imported cases of canine angiostrongylosis have been reported (Maier *et al*., 2010) up to now, but one of the seropositive dogs found during our survey came from the Lower Austrian village of Deutsch Jahrndorf on the Austria-Slovakia-Hungarian border. To the best of our knowledge, no data about the occurrence of *A. vasorum* in Ukraine (bordering eastern Slovakia) are available. Most recently, an autochthonous case of canine angiostrongylosis was diagnosed is Serbia (Simin *et al*., 2014) and it can be assumed that further cases will occur.

Further monitoring is required in order to evaluate the distribution of this parasitic disease in Slovakia. However, as our experience indicates, newly emerging diseases are associated with great difficulties such as determining the correct clinical diagnosis, the use of appropriate diagnostic tools and, correspondingly, the provision of adequate treatment by veterinary practitioners (Miterpáková *et al*., 2012). Efforts to increase awareness of this potentially fatal parasitic disease among animal owners and veterinary practitioners are therefore needed.

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